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DIAGNOSIS, TREATMENT AND EVALUATION OF CHRONIC VENOUS DISEASE

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DIAGNOSIS, TREATMENT AND EVALUATION OF CHRONIC VENOUS DISEASE

THESIS FOR DOCTORAL DEGREE (PH.D.)

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To my family

"You can't ever reach perfection,

but you can believe in an asymptote

toward which you are ceaselessly striving".

Paul Kalanithi

Neurosurgeon and writer

Passed member of Stanford University Hospital

ABSTRACT

BACKGROUND AND AIMS

Varicose veins (VVs) are the most common manifestation of chronic venous disease (CVD) in the Western hemisphere. Recent research shows that the disease encompasses the global population and with similar prevalence. The most severe form of CVD is venous ulceration with a prevalence of about 1% that does not only cause great suffering but is also costly for the society. The aims of this thesis were to investigate ways in improving prioritization, diagnosis, and surgical treatment of patients with VV that have the potential risk of developing VU.

METHODS:

Cross-sectional (Studies I, III and IV) and follow-up (Study II) studies were carried out. Study I investigated the inter-observer reproducibility of the clinical class of CEAP and whether there was a medical indication for treatment. Seventy-eight patients (106 limbs) with varying degree of CVD were included. Three independent physicians assessed the patients. In study II, we assessed the ulcer recurrence rate in patients with healed or active venous ulcer (VU) that were treated with endovenous laser ablation (EVLA) for superficial venous incompetence. One hundred and seventy patients (195 limbs) of consecutively treated patients were followed-up. Study III investigated the association between the biomarker E-XDP in plasma and the presence or severity of CVD. Samples of blood were drawn from a total of 142 patients with CVD and were matched to VU patients. In Study IV, 112 patients with CVD were included. The VEINES-QOL/Sym questionnaire was translated into Swedish and evaluated with regards to its psychometric properties.

RESULTS

In **Study I**, total agreement between the three observers for clinical class was obtained in 61% of all cases ($\varkappa$ 0.55-0.68 (95% CI)) and for medical indication 60% of all cases ($\varkappa$ 0.35-0.57 (95% CI)). **Study II** showed that all patients had healed their original ulcer and 84% of the limbs had no recurrence after 3.5 years. In **Study III,** E-XDP levels were elevated in patients with CVD compared with controls ($p< 0.05$) and increased with increasing disease severity ($p = 0.02$). Multiple linear regression confirmed that E-XDP was independently associated with CVI ($p < 0.05$) after adjustment for age and gender. **Study IV** showed excellent internal consistency for both VEINES-QOL (Cronbach's alpha (α) = 0.93) and VEINES-Sym ($\alpha = 0.89$). Both the VEINES-QOL and VEINES-Sym correlated well to all the RAND-36 domains, demonstrating good construct validity. Exploratory factor analysis confirmed both subscales of the VEINES-QOL/Sym.

CONCLUSIONS

Diagnosis using the clinical class of CEAP has moderate reproducibility when deciding medical indication for treatment and EVLA in VU patients achieves good healing with low recurrence rates and low rates of complications. Further, the novel biomarker E-XDP appears to have a positive association with increasing disease severity. The Swedish version of the VEINES-QOL/Sym is valid in assessing health related quality of life in CVD, both clinically and in research.

LIST OF SCIENTIFIC PAPERS

I. Inter-observer variability in the assessment of the clinical severity of superficial venous insufficiency
Sinabulya H, Holmberg A, Blomgren L.
Phlebology, 2015, vol. 30 no. 1, 61-65

II. Mid-term outcomes of endovenous laser ablation in patients with active and healed venous ulcers: A follow-up study
Sinabulya H, Östmyren R, Blomgren L.
Eur J Vasc Endovasc Surg, 2017, E-pub ahead of print
DOI:http://dx.doi.org/10.1016/j.ejvs.2017.02.028

III. Cross linked fibrin degradation products in plasma are elevated in chronic venous disease
Sinabulya H, Silveira A, Blomgren L, Roy J.
In manuscript.

IV. Cultural adaptation and validation of the Swedish VEINES-QOL/Sym in patients with venous insufficiency
Sinabulya H, Bergström G, Hagberg J, Johansson G, Blomgren L
Submitted.

CONTENTS

LIST OF ABBREVIATIONS

AASV	Anterior accessory saphenous vein
ABPI	Ankle brachial pressure index
ANOVA	Analysis of variance
CEAP	Clinical-Etiology-Anatomy-Pathophysiology
CVD	Chronic venous disease
CVI	Chronic venous insufficiency
DUS	Duplex ultrasound scanning
DVT	Deep venous thrombosis
ELISA	Enzyme-linked immunosorbent assay
EVLA	Endovenous laser ablation
GSV	Great saphenous vein
HL	High ligation
HRQoL	Health related quality of life
Ms	Millisecond
PPG	Photoplethysmography
PROM	Patient reported outcomes
PTS	Post-thrombotic syndrome
PVI	Perforator vein insufficiency
RAND-36	RAND 36-item health survey
RFA	Radiofrequency ablation
SD	Standard deviation
SSV	Small saphenous vein
SVI	Superficial venous insufficiency
UGFS	Ultrasound guided foam sclerotherapy
VEINES-QOL/Sym	VEnous Insufficiency Epidemiological and Economic Study on Quality of Life/Symptoms
VU	Venous ulcer
VV	Varicose vein
XDP	Cross-linked fibrin degradation products

INTRODUCTION

HISTORICAL PERSPECTIVE

The knowledge about varicose veins (VV) dates from the late fifth century BC and early fourth century BC when Hippocrates first described not only VV but also their correlation with leg ulcers (Figure 1)(1). Later on in the 16th century the French surgeon Ambroise Paré and Italian surgeon and anatomist Gabrielle Fallopio theorized that VV and leg ulcers secondary to VV were due to bad humors that if not treated would progress into serious illness (2).

Treatment of varicose veins has also evolved over the years with the understanding of the cause of the disease. When varicose veins were treated during Hippocrates times, he recommended the use of bandages on the leg to produce firm compression and advised small punctures in varicose veins(1, 3). Surgical treatment with the today gold standard, high ligation and division and later addition of surgical stripping was first described in the early 20th century (3). The addition of stripping was to reduce the recurrence rates of varicose veins. Consequent treatments including phlebectomies, perforator vein surgery and most recent, minimally invasive procedures have evolved and become increasingly important(3).

Figure 1. *Votive plate from Athens, Greece. Shows a man holding a huge leg with varicose vein.*

DEFINITIONS

Chronic venous disease (CVD) as defined by the VEIN-TERM consensus document is "(Any) morphological and functional abnormalities of the venous system of long duration manifested either by symptoms and/or signs indicating the need for investigation and/or care"(4). Another commonly used term is chronic venous insufficiency (CVI), referring to advanced CVD that involves skin changes and venous ulceration(4).

EPIDEMIOLOGY

Varicose veins is the most common manifestation of CVD in the Western hemisphere with a prevalence that varies depending on the study at hand but can be approximated to 25%-33% in females and 10%-40% in male subjects(5, 6). The prevalence of CVD has also been shown to be a universal condition and not limited to the western world(7). The prevalence of CVI is 7% for skin changes such as eczema, hyperpigmentation and lipodermatosclerosis and for the most advanced form, venous ulceration, 1%(6, 7). It is not known how common VU is globally but a study conducted in Brazil showed that VUs were more common in people with dark skin(8). Venous ulcers (VUs) are often painful, longstanding and entail high staff expenses due to the frequent need of dressing change and compression treatment (9). Overall annual costs of CVD represent 1–2% of the total healthcare budget in Western European countries and in the USA (10, 11).

PATHOPHYSIOLOGY

To better understand the pathophysiology of CVI it is important to first understand the normal anatomy and physiology of lower-extremity veins. There are two vein systems in the lower extremity, a superficial and deep vein system that are divided into compartments by the muscular fascia and connected by perforating veins (Figure 2) (12).

The veins of the superficial compartment drain the cutaneous microcirculation and include the great saphenous vein (GSV), small saphenous vein (SSV) and numerous tributaries(13).

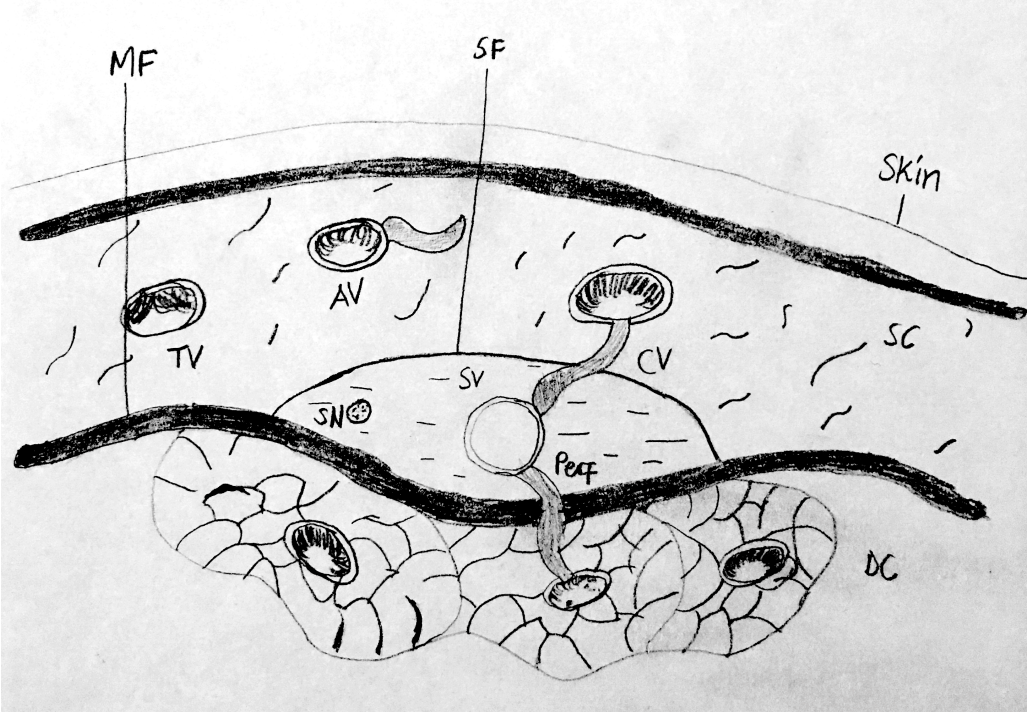

Figure 2. *Deep and superficial venous compartments. Muscular fascia (MF) separates the superficial compartment (SC) from the deep compartment (DC). The superficial compartment contains saphenous veins (SV), tributary veins (TV), and accessory veins (AV). Saphenous veins and accompanying nerves (SN) are contained within a saphenous compartment that is bound superficially by saphenous fascia (SF) and deeply by muscular fascia. The deep compartment is bound by muscular fascia and contains the deep veins. Perforator veins (Perf) traverse the superficial and the deep compartments. Communicating veins (CV) connect veins within the same venous compartment, either deep to deep or superficial to superficial. Illustrated by Juliet Sinabulya*

The vein wall unlike in arteries is thin and transports blood from the periphery to the heart (14). With the assistance of the muscle pump in the extremities, blood flows in a forward direction and bicuspid valves in the veins prevent retrograde flow (reflux) (Figure 3) (15).

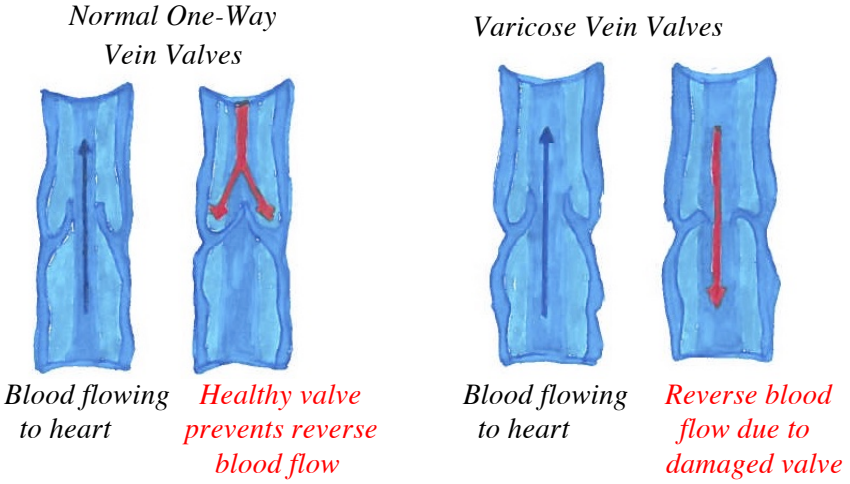

Figure 3. *Normal and pathological vein structure. Illustrated by author.*

The pathophysiology of CVI is characterized by increase in venous pressure impairing the return of blood flow to the heart(16). This is a result of either reflux, obstruction or both and is exacerbated by muscle pump dysfunction in the lower extremity(14). Reflux may either be of primary or secondary etiology. Primary CVI is considered to be a result of an underlying connective tissue defect perceived as valvular incompetence or destruction and dilated veins leading to increased ambulatory venous pressure(5, 17).

Secondary CVI may be due to previous deep vein thrombosis (DVT), with an annual incidence of 0.1% and affects 2-5% of the population in a lifetime (18). An estimate of 25-50% develop a complication to DVT called post-thrombotic syndrome (PTS), resulting from incomplete lysis of thrombus that causes fibrosis and obstruction that may destroy the vein valves (19). PTS may present as CVI including skin changes and venous ulceration (20).

At the microcirculation level; venous hypertension, mechanical injury or stress due to the vein structure induces capillary leakage allowing the accumulation of several circulating products such as fluids, red blood cells and protein(16). Degradation of red blood cells and protein extravasations initiate an inflammatory reaction resulting in leukocyte migration leading to the complications ranging from edema and lipodermatosclerosis to tissue necrosis and venous leg ulceration (Figure 4)(21).

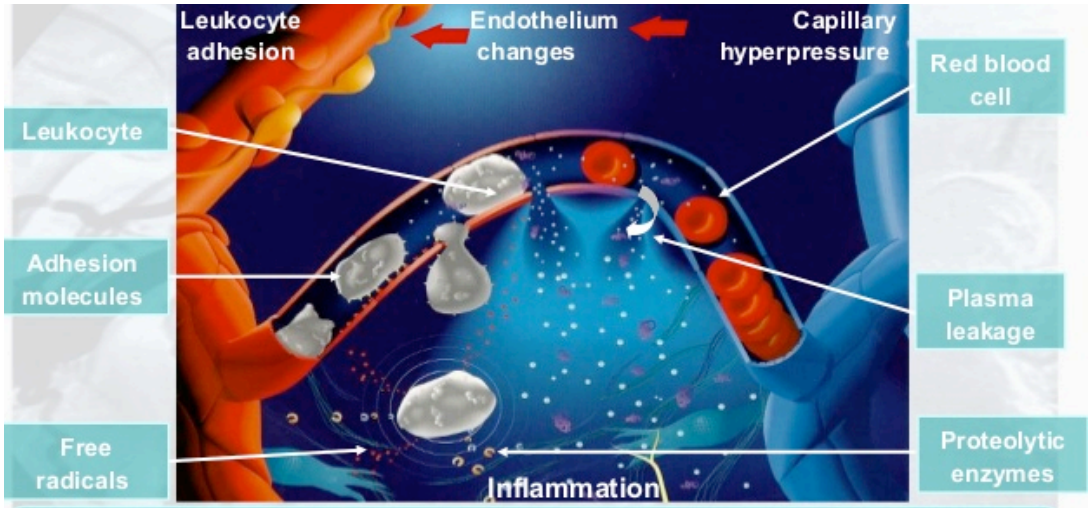

Figure 4. *Leukocyte adhesion and migration due to venous hypertension. Reproduced with permission from Dr Fatih Islamoglu, Turkey.*

VENOUS ULCER

Venous ulceration being the most severe form of CVD, resulting in a lot of pain and suffering plus great costs to society, renders much attention. It is still unclear why some people develop VUs secondary to VVs (primary CVI) or DVT (secondary CVI), thus making the clinical diagnosis more complex and difficult to predict which patients are at a higher risk of developing venous ulceration.

Studies on VU have shown that chronic inflammation is the main cause(17, 22, 23). One theory being through leukocyte activation that damages connective tissue and the skin in the leg(24). Another is interepithelial pore widening that allows the deposition, of fibrin and other macromolecules that would otherwise aid in wound repair, to the dermis(25). The pathophysiology behind the inflammation on a microcirculation level is as described under Pathophysiology and can be summarized as secondary to persistent elevation of ambulatory venous pressure(26).

The presentation is normally over bony prominences, particularly in the gaiter area (ankle to mid-calf) and they are generally irregular, despite well defined borders, shallow with red granulation tissue and fibrin slough (yellow-white exudate) in the ulcer base(27). Another common presentation is the "champagne bottle deformity" perceived as contraction of the subcutaneous tissues of the gaiter area (Figure 5) (23).

Figure 5. *Chronic VU characterized by inflammation surrounding the ulcer, irregular ulcer border, and wound bed with granulating matrix. There is excess drainage from the venous leg ulcer rich in cytokines and proteinases that exacerbate a condition of inflammation. You can even see tendency to "champagne bottle deformity". Photo by Lena Blomgren*

The baseline treatment of VU for thousands of years has similarly to VVs been compression (28). However, well-conducted randomized trials have shown that surgical treatment of VV complemented with compression reduces the risk of ulcer recurrence (29, 30). Other treatment forms include topical treatments such as silver sulphadiazine, cadexomer and oasis matrix, all studied in randomized trials and shown to improve ulcer healing(23). Even anti-inflammatory drugs have shown in several studies to improve microcirculation of the leg and thus aiding in the healing process(27).

Biomarkers for Chronic Venous Disease

Due to the gap in knowledge on etiology of VU whether secondary to VV or DVT, we seek methods to help improve prioritization when it comes to patient selection for surgery. Finding a biomarker with direct association with CVI could become a possible predictor of disease(31).

We have understood that inflammation almost certainly plays a role in CVI but there are few studies that have evaluated the direct association between specific biomarkers and venous disease(22). We also know that venous ulceration involves coagulopathy including thrombophilia but the causal relationship between VU and coagulopathy remains difficult to clarify(32, 33).

Smith et al reviewed the current literature on the association of circulating markers with primary CVI and identified three that were linked to alteration of vascular wall homeostasis; homocysteine, vascular endothelial growth factor (VEGF) and estradiol (34). Homocysteine is realized as a biomarker that increases endothelial-leukocyte interaction and venous thromboembolism development(35, 36). VEGF affects vascular tone and when plasma levels were examined in correlation with clinical class they have been shown to have a positive trend towards higher levels compared to controls but with statistical significance only in patients with healed ulcers(37). Elevated serum levels of estradiol, which too affects vascular tone, in both men and women have also been shown to be associated with VV(38, 39). Another well studied thrombolysis-related and clinically used biomarker in patients with venous thromboembolism is D-dimer(40). It is elevated and widely used clinically in many diseases such as DVT, pulmonary embolism and more(41, 42).

A novel biomarker for the activation of leukocytes, cross-linked fibrin degradation products (XDP), has been shown to be elevated in patients with venous thromboembolism but there is no data on levels in patients with CVI(40, 43). XDP is produced specifically when neutrophil elastase breaks down fibrin that is present in thrombus (Figure 6) (44). This is of special interest in the development of venous ulceration as this involves both thrombosis and the activation of leukocytes.

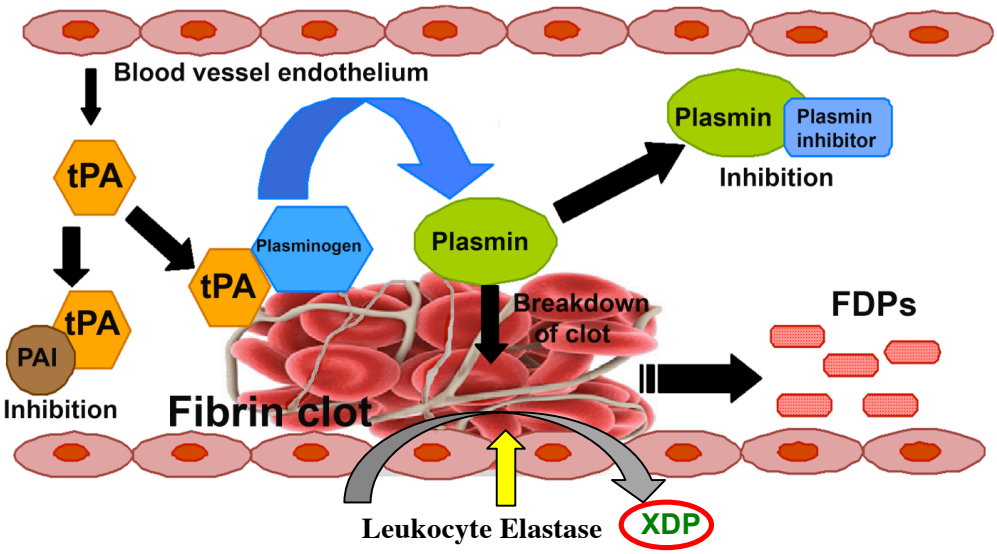

Figure 6. *Tissue plasminogen activator (tPA) activates Plasminogen to Plasmin that cleaves fibrin, forming fibrin degradation products (FDPs). Plasminogen activator inhibitors (PAIs) are elevated during inflammation. Leukocyte elastase, released from activated leukocytes, digests fibrin yielding cross-linked FDPs (XDP) that are different from those yielded from Plasmin. Adapted from Bhattacharjee et al ©(45).*

D-dimer is also a fibrin degradation product that unlike elastase mediated XDP is a digest of Plasmin(46). A draw back of this marker is its lack of specificity in positive cases of venous thromboembolism(47). Thus the need of a more specific biomarker with regards to CVD.

All in all, it is suggested that the pathogenesis of CVI is multifactorial and complex and that biomarker research aids the clinician by improving the current knowledge on prognostic information on CVI(34).

DIAGNOSIS

CLINICAL EXAMINATION

The American Venous Forum met in Hawaii, 1994 and agreed upon a consensus document to be used for the classification of chronic venous diseases and a scoring system of the severity of CVD(48). The CEAP classification (Clinical-Etiology-Anatomy-Pathophysiology) was formed and is widely used clinically and in research(49). It was revised in 2004, creating the basic CEAP (Table 1) and is today gold standard for classification of CVD(50, 51). Nonetheless the CEAP has been shown to have a moderate inter-observer reproducibility(49).

The CEAP classification provides a descriptive tool for patients with CVD but has also been critiqued for lack of consistency with common symptoms such as pain and lack of responsiveness when evaluating patients in the long term(52, 53).

Venous Clinical Severity Score (VCSS) is the scoring system also included in the consensus document above(48). The VCSS is one of three venous severity scores that grade the clinical symptoms of venous disease. The other two are Venous Segmental disease score (VSDS) that grades anatomic and patho-physiologic symptoms and Venous Disability Score (VDS) which is an extension of CEAP and evaluates the ability to work(54). The most widely used is VCSS, complementing the CEAP and correlates to health related quality of life (HRQoL) measures(55).

In conjunction with clinical examination, established risk factors such as age, female gender, multiparity, family history, obesity, and occupations associated with prolonged standing should be included(56).

Table 1. *Clinical class of CEAP (Clinical-Etiology-Anatomy-Pathophysiology)*

C class	Description
Class 0	No visible or palpable signs of venous disease
Class 1	Telangiectasies, reticular veins, malleolar flare
Class 2	Varicose veins; diameter ≥ 3 mm
Class 3	Edema without skin changes
Class 4	Lipodermatosclerosis or atrophie blanche
Class 5	Skin changes with healed ulceration
Class 6	Skin changes with active ulceration

Adapted from Eklöf et al. (50)

DUPLEX ULTRASOUND

In addition to physical examination of patients with CVD, it is also recommended that duplex ultrasound scanning (DUS) of both the deep and superficial veins be performed as the primary diagnostic method(57). The method is accurate, safe and non-invasive and if performed as routine preoperative investigation, it has been shown to improve surgical results of uncomplicated VV(58). With DUS you can achieve information about flow and hemodynamic parameters such as refilling time, velocity at peak reflux, vein diameter and volume flow(59).

Reflux is considered significant if longer than 500 ms for superficial veins and for popliteal and femoral veins the cut-off is 1 second(57). This is the time required by the initial reflux of blood to bring normal valve leaflets into apposition. The official definition of perforator vein reflux is if longer than 500 ms but this doesn't necessarily mean pathological significance and in clinical practice pathology is considered if the perforating vein is dilated with outward flow and in near vicinity to skin changes or VU(60-62).

DUS is also used in the diagnosis of DVT(63). One should note though that when examining the deep veins of the lower leg, DUS has been shown to have a lower reliability on investigating patency, obstruction or occlusion(64). However, the reverse was shown for the femoro-popliteal veins(65). When DUS was compared to air plethysmography, another non-invasive technique used in evaluation of CVI, there was good reproducibility of measurements by the two techniques(66).

PHOTOPLETHYSMOGRAPHY

A complementary examination to DUS in the diagnosis of CVD is photoplethysmography (PPG), a method in which the transmission of light reflection in the subdermal venous plexus is detected as a measure of the change of blood volume in the skin(67). The advantage of PPG is that it's a non-invasive, cheap, simple and sensitive test suitable for outpatient use(68). It offers a quantitative measure of venous reflux (refill time) as a complement to DUS, which on it's own shows the anatomical distribution of VV(69, 70).

As PPG doesn't have good relation between refill time and venous disease severity, DUS remains superior to PPG as it gives more information than refilling time as

explained under "Duplex Ultrasound"(71). The combination of PPG, clinical examination and DUS could possibly improve the prioritizing of VV patients.

PRIORITIZATION

Several studies have shown that there is a correlation between venous hypertension and CVD symptoms and signs including heaviness, swelling, aching and cramps but this association has been inconsistent between patients(72-74). This additionally complicates clinical practice when predicting which patients with VV are at risk of developing a VU in future. We know that surgical treatment of VV in patients with VU reduces the risk of ulcer recurrence(29).

The current recommendations in several Swedish county councils allow reimbursement for surgery in cases of VU, skin changes and where severe symptoms affect HRQoL negatively. Although there is a correlation between venous hypertension and CVD signs and symptoms, several studies have shown that there is a poor correlation between leg symptoms and extent of superficial venous pathology on DUS(74, 75). This may lead to difficulties in patient assessment for the treating surgeon in the case of a patient with severe symptoms but no skin changes resulting in a conflict of opinion on whether the surgery should be reimbursed by the county council or not(57).

The clinical class C of CEAP is the basis for the Stockholm county council's recommendations on reimbursement, but as mentioned previously, it was last revised in 2004 and has been shown to have a moderate inter-observer reliability(49, 76). Another issue is allocation of government resources to the care and treatment of VV patients where it has been shown that VV wait for their surgery longer than gallstones or inguinal hernia (77). This may also be explained by the fact that VV often have low priority and have been referred to as the "Cinderella" of surgery(78).

QUALITY OF LIFE MEASURES IN VENOUS DISEASE

The most severe form of CVD, VU is both costly for society and painful for the patient (9). As many as 10% of the adult British population suffers from depression at any one time and depression is twice as common in CVD patients (79). It has been shown that VV without skin changes cause a reduced quality of life comparable to gallstones and inguinal hernia (80). According to the European and

American clinical practice guidelines, the assessment of CVD should also include quality of life evaluation (53, 57).

Currently there are several generic and disease-specific instruments used to measure HRQoL, clinically and for research purposes. Among the widely used generic instruments are the Short Form 36-Item Health Survey and EuroQoL-5D (EQ-5D)(81). Some of the most popular and validated disease-specific instruments are the Aberdeen Varicose Veins Questionnaire (AVVQ), Chronic Venous Insufficiency Questionnaire (CIVIQ) and the VEnous Insufficiency Epidemiological and Economic Study on Quality of Life/Symptoms (VEINES-QOL/Sym) (55).

The AVVQ is sensitive in assessing functional outcome after treatment for chronic venous disease and CIVIQ is a tool created for C0 - C4 patients and unlike the AVVQ, it takes into account the more psychological effects on quality of life(82). VEINES-QOL/Sym evaluates quality of life and symptoms for the whole spectrum of venous disease, from telangiectasies to skin changes and VU(55).

Even with several validated disease-specific HRQoL instruments in many languages, there is no such instrument for CVD in Swedish, and it is needed both for research purposes and for clinical evaluation.

TREATMENT IN CHRONIC VENOUS DISEASE

There are a variety of treatment methods available for patients with CVD and the commonly recommended treatment of VVs is compression but with a compliance that varies between 30 % and 65% (83). The gold standard treatment for VVs in the GSV has been surgery with high ligation (HL) and division at the confluence of the GSV and the femoral vein and the addition of stripping has either been performed consequently or later (3). VVs in the SSV have also traditionally been treated with HL at the confluence of the SSV and popliteal vein (84).

During the last decade minimally invasive techniques such as Endovenous Laser Ablation (EVLA), Radiofrequency Ablation (RFA) and Ultrasound-guided Foam Sclerotherapy (UGFS) have gained popularity over conventional surgery (85). These techniques have the advantage of being able to be performed in local anesthesia and thereby allowing even elderly patients with comorbidities treatment (86). Even with this knowledge, clinical experience shows that few patients with VU are referred directly for surgical intervention upon presentation; rather they are

often treated conservatively for years with recurring ulcers before referral. The reason for this is uncertain but one may speculate that the physicians who these patients often first seek help from are not aware of current guidelines or that they believe that the ulcer must first heal before consideration for surgery and that surgical treatment may be too invasive for this patient group.

When EVLA and RFA have been compared to conventional surgery, studies have shown that it is as effective (87). In clinical practice EVLA and RFA are increasingly used to treat patients with VU and there are several reports about low recurrence rates but with small groups and short follow-up(88),(89).

AIMS OF THE THESIS

RATIONALE

In this thesis, aspects of diagnosis, treatment and evaluation of chronic venous disease (CVD) are studied. VU the most severe form of CVD is a great burden both for the society as well as for the diseased patients.

The American and European venous disease guidelines have been formed to guide in diagnosis and treatment and create a broad basis for further research. One much needed area of research is biomarkers and CVI, that have and are currently being studied. The question being; is there a need for a novel biomarker within the field of CVD. Health related quality of life scoring is another recommendation for evaluation of CVD; is there a need for a scoring questionnaire in Swedish?

OVERALL AIM OF THE THESIS

To investigate ways in improving prioritization, diagnosis, and surgical treatment of patients with VV that have the potential risk of developing VU. This has been done by way of four studies.

SPECIFIC AIMS

- Study I: To test the inter-observer reproducibility of the clinical class of CEAP when used in a clinical setting where the patients' clinical signs and symptoms were evaluated if severe enough for treatment within the national health insurance system.
- Study II: To assess the ulcer recurrence rate after a longer follow-up in a larger cohort of patients with healed or active VU treated with EVLA for superficial venous incompetence (SVI) and to search for possible risk factors for non-healing or recurrence.
- Study III: To investigate the association between the biomarker E-XDP in plasma and the presence or severity of CVD.
- Study IV: To translate and evaluate the psychometric properties of the VEINES-QOL/Sym in a Swedish cohort of patients with venous disease.

PATIENTS AND METHODS

OVERVIEW OF STUDY DESIGN

In Table 2 is a summary of the study design, population and statistical methods in this thesis.

Table 2. *Overview of studies*

Study	Design	Study population	Statistics
Study 1	Cross-sectional cohort study	78 Consecutive patients 106 limbs	Kappa-analysis
Study II	Follow-up study	170 patients, 195 limbs	Chi-square, Fishers exact test and Students t-test Logistic regression analysis
Study III	Cross-sectional cohort study	142 patients matched for age, gender and C class	Students t-test and ANOVA Multivariate linear regression analysis
Study IV	Cross-sectional cohort study	112 Consecutive patients	Exploratory factor analysis Reliability and correlation analysis

ANOVA; Analysis of variance

PATIENTS

All patients included were recruited from Venous Centre (VC), a high volume centre in Stockholm, Sweden, dedicated to the treatment of varicose veins. In all but study II, consecutive patients were asked to participate and in study II patients were identified from the medical records and quality registry. In study III included patients were matched between the C-classes and those graded as C-class 0 or 1 and without history of DVT were used as controls.

At the time of the studies, VC treated patients who were reimbursed by the Stockholm County or by insurance companies, and also patients who paid for the

treatment themselves. The clinic treated all C classes and were reimbursed for classes C3-6 with the addition of patients with nonspecific symptoms such as heaviness, tingling and pain if (*i*) there was no other etiology to the mentioned symptoms, (*ii*) the HRQoL was clearly affected, (*iii*) treatment with compression relieved symptoms and (*iv*) if significant reflux was seen on DUS, Doppler or any other quantitative investigation of the venous system (Table 3).

Upon inclusion, all patients were graded according to the C class of CEAP classification and in studies II-IV history of DVT was also asked.

A drawback with using a private clinic for research that receives reimbursement from the local county is that during certain periods of the year, very few if any patients with CVI would be treated. This due to need for annual renegotiations of reimbursement fees that could at times take months and after which would result in less fees than actual patient load.

ETHICAL CONSIDERATIONS

The Regional Ethical Review Board in Stockholm approved all the studies. All patients received written information and signed a consent form before participation.

In Sweden, many CVD patients are now treated in private clinics, so for research purposes it was easier to recruit patients from such a centre. In study I and II, patient inclusion was not as difficult since these patients sought initial care or follow-up after treatment. In study III and IV there was more of a challenge due to the nature of the studies that may be more difficult for the patient to perceive the direct importance at their actual time of consultation. When it came to patient recruitment of controls in study III, this proved to be the biggest challenge as these were perfectly healthy patients with minor or if any physical symptoms being asked to take extra time and to undergo, even if minor, an invasive investigation.

METHODS

STUDY I

The study took place between August 2011 and February 2012. All patients were evaluated with regards to whether there was a medical indication for treatment

according to the Stockholm County guidelines, set in 2005 (Table 3). Exclusion criteria were: patients not willing to participate in the study, CEAP classification done previously, language problems or dementia.

Three physicians, consisting of a surgical resident, a consultant vascular surgeon from a university hospital and a consultant vascular surgeon from the private clinic, assessed the patients independently and their respective assessments were blinded to each other. The assessment was with regard to C class of each patient and whether there was a medical indication for treatment. This choice of having physicians with different backgrounds and levels of experience was intentional as it better reflects the every day care of CVD patients all over the nation. Another option could have been to invite a general physician to partake or a general surgeon who do not treat VV patients but encounter such cases on a day-to-day basis.

Upon examination the patients were given license to describe their own symptoms and were examined by each physician to see if there was a medical indication for treatment according to the Stockholm County guidelines. DUS was performed on all patients to confirm the presence of superficial venous insufficiency, and to exclude patients with post thrombotic changes.

Table 3. *Distinctions between medical and cosmetic indication for treatment of VV by the expert surgical committee in Stockholm County 2005-12-01*

Grade	Symptoms	Medical Indication
1	Venous ulcer	Yes
2	Venous eczema /stasis pigmentation	Yes
3	Edema, thrombophlebitis, VV with bleeding	Yes
4	Nonspecific symptoms (heaviness, tingling, pain, etc.)	Yes*
5	VV without symptoms (cosmetic)	No
6	*Telangiectasies (cosmetic)*	*No*

** If no other etiology to symptoms, HRQoL clearly affected, if treatment with compression relieves symptoms and if significant reflux on DUS, Doppler or any other quantitative investigation of the venous system.*

STATISTICAL ANALYSIS

Sample size was calculated to a minimum of 51 limbs with three raters if we wished to demonstrate that the reliability coefficient $\varrho > 0.4$ with 80% power for a

significant result at α=0.05 when ϱ=0.6(90). Cohen's kappa, *k*, and weighted kappa were used when analysing agreement between pairs of examiners for C of CEAP. Kappa agreement was interpreted as slight (ϰ = 0.0–0.20), fair (ϰ = 0.21–0.40), moderate (ϰ = 0.41–0.60), substantial (ϰ = 0.61–0.80), and almost perfect (ϰ = 0.81–1.0)(91). Fleiss kappa, *k*, was used to measure the inter-observer agreement between all three examiners for both C of CEAP and medical indication(92).

STUDY II

In this study recruitment was between April 2013 and February 2015. The main treatment methods at the clinic at time of the original treatment were EVLA, ambulatory phlebectomies and UGFS.

Patients treated with EVLA for SVI, and who at the time of treatment had a healed or open VU, clinical class C5 and C6 were invited to participate in the study. Figure 2 shows the flow of patients.

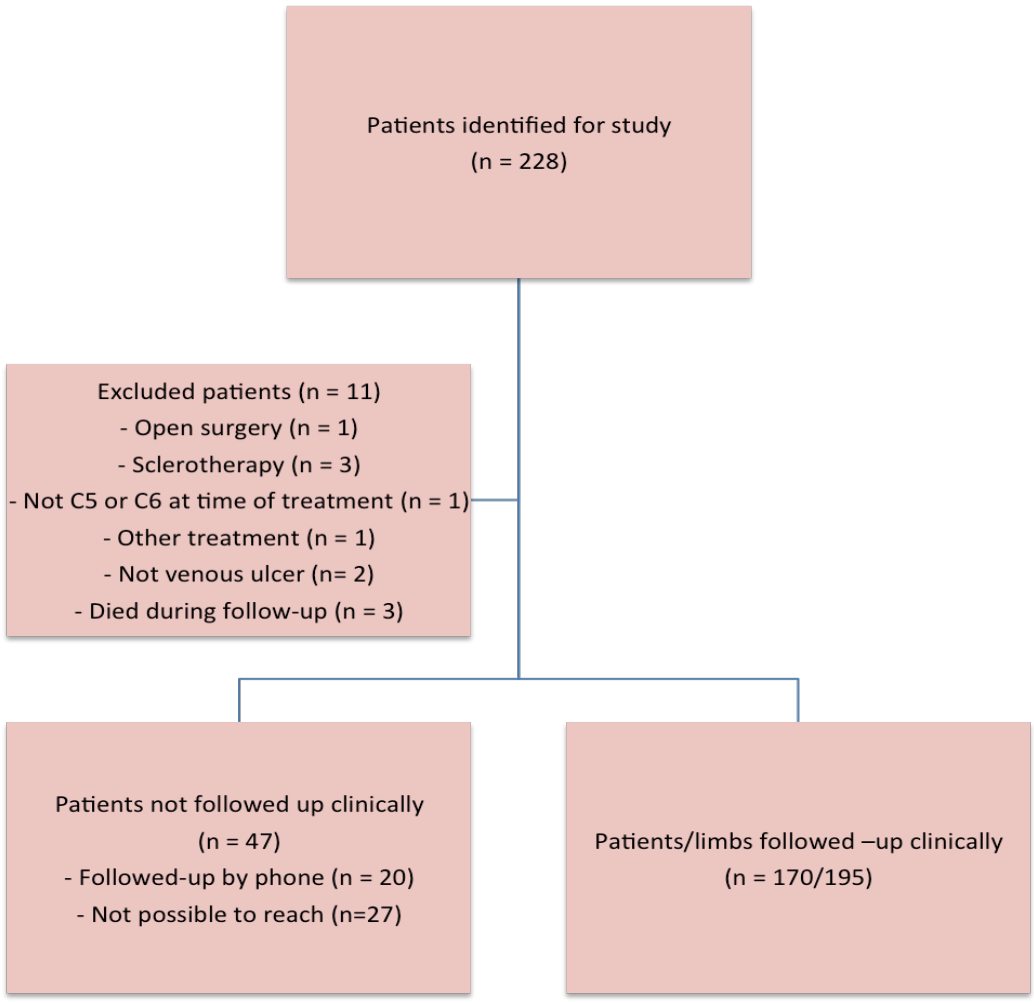

Figure 2. *Chart showing the flow of patients through the study*

At the follow-up examination medical history was taken from the patient including several risk factors and postoperative complications such as DVT, infection and nerve injury. Generic HRQoL scoring was measured using the (EQ-5D)(93). Ankle brachial pressure index (ABPI), PPG, DUS and evaluation with venous clinical severity score (VCSS) were also performed.

STATISTICAL ANALYSIS

Chi-square, Fisher's exact test and t-tests were used to compare proportions between the original C5 and C6 groups, and to compare patients with and without recurrent VU at follow-up. Level of statistical significance was considered $p < 0.05$. Logistic regression was used in the multivariate analysis to identify independent risk factors for ulcer recurrence.

STUDY III

Patient recruitment was between January 2015 and April 2016. Included patients were asked about any current medication with a statin or anticoagulant. Thereafter samples of peripheral blood were drawn preceding surgical treatment.

ENZYME-LINKED IMMUNOSORBENT ASSAY

The blood samples taken were used to measure plasma levels of cross-linked fibrin degradation products by leukocyte elastase (E-XDP). This was done using a sandwich enzyme-linked immunosorbent assay (ELISA) as described by Kohno et al(94). The monoclonal antibody anti-E-XDP clone IF-123 (Cosmo Bio Co. Ltd., Tokyo, Japan) was used as capture antibody since it specifically recognizes elastase-digests of human fibrinogen and fibrin, but not their plasmin-digests(95). Horseradish peroxidase-labeled anti-human fibrinogen rabbit antibody (DAKO, Glostrup, Denmark) was used as probing antibody.

For further characterization of the antibody IF-123, we incubated fibrinogen (3mmol/L) with elastase (Product E-8140, Sigma), plasmin or reaction buffer alone (0.05M Tris, 0.15M NaCl, pH 8.0), proteases at 88 nmol/L. Samples were taken with minutes intervals for 1 hour and treated with excess of protease inhibitor cocktail (complete, mini, EDTA-free, from Roche). Samples were thereafter analyzed for reactivity in the ELISA described above. Incubation of fibrinogen with

elastase generated product(s) that reacted in the ELISA with IF-123 (seen as increase in optical density, in the samples taken after 40 minutes incubation), which did not occur with incubation with plasmin or buffer alone.

STATISTICAL ANALYSIS

The binary logarithm of E-XDP concentration (simply referred to as E-XDP) was used in all analyses. Differences of means between two groups were examined for statistical significance using the Student's *t*-test for independent samples. A *p*-value less than 0.05 denoted the presence of a statistically significant difference. Multiple linear regression was used to study the association between plasma E-XDP concentrations and severity of CVD.

STUDY IV

Patients with varying degrees of CVD were recruited in May 2016. Data was collected including age, gender and history of previous VV surgery. All patients were provided with a copy of both the Swedish VEINES-QOL/Sym and the RAND 36-item health survey (RAND-36) to complete.

TRANSLATION AND TESTING

The questionnaire was translated into Swedish with forward-backward translation as described by Beaton et al. (96). Three researchers with special interest in Phlebology were involved in the total process. The resulting Swedish VEINES-QOL/Sym (VEINES-QOL/Sym-Swe) became the final version administered to the study population and used to evaluate reliability and validity.

STATISTICAL ANALYSIS

Feasibility of the questionnaire was assessed by the overall response rate. Floor and ceiling effects were checked (i.e., response categories with high endorsement rates at the bottom/top ends of the scale, respectively) for summary scores. Descriptive baseline characteristics were presented as means and standard deviations (SD) or numbers and percentages (%) as appropriate. A two-sided p value of <0.05 was considered statistically significant.

Reliability in the context of health outcome measurement is the ability of the instrument to measure its subject consistently. This was tested using the reliability coefficient Cronbach's alpha (α) that assesses internal consistency. Structure of the VEINES-QOL/Sym was tested using exploratory factor analysis. Factor analysis is a statistical method that is used to explain whether or not the pattern of responses on a number of items can be explained by a smaller number of underlying factors(97). Good structure was if items loaded a value higher than 0.35.

Construct validity assesses whether the scales scores behave as predicted according to existing knowledge about the population and the disease and involves comparisons with other variables. Convergent validity that is a form of construct validity tests whether the score has positive relationships with other quality of life and well-being scales. This was tested using the Spearman's rank correlation coefficient. Another adjunct to construct validity is known-group differences that are used to compare two groups that are known to have certain results from previous studies.

RESULTS

STUDY I

- The reproducibility of C when deciding medical indication for treatment is moderate.

The majority of the included patients (85.9%) were female and the median age of the study-patients was 58.5 years (range= 23-91). The C-class distribution of patients for each respective observer is shown in Table 4.

Table 4. *Number of limbs in each C class for each respective observer and number in total agreement*

C class	Observer 1	Observer 2	Observer 3	Total Agreement	Kappa	Z	P(vs > 0)
0	2	3	3	2	0.74	13.3	<0.001
1	20	21	18	16	0.82	14.6	<0.001
2	43	38	48	30	0.57	10.2	<0.001
3	5	7	6	0	0.17	3.1	<0.001
4	28	39	24	14	0.56	10.0	<0.001
5	3	2	2	1	0.71	12.6	<0.001
6	2	3	2	2	0.85	15.2	<0.001

The numbers in Table 4 are similar; however, the numbers in each class do not necessarily represent the same patients. Least agreement was seen for class C3, followed by C2 and C4. The agreement between pairs of examiners for all clinical classes of CEAP is shown in Table 5.

Table 5. *Exact agreement between examiners for C class of CEAP*

Observers	Agreement (%)	95% CI	Wκ (95% CI)
All	*61.3*	51.4-70.6	
1 vs 2	*78.3*	69.2-85.7	0.82 (0.73-0.92)
1 vs 3	*67.9*	58.2-76.7	0.76 (0.65-0.87)
2 vs 3	*70.8*	61.1-79.2	0.75 (0.63-0.86)

CI = confidence interval; Wκ = weighted kappa.

The agreement between pairs of examiners concerning medical indication for treatment is shown in Table 6.

Table 6. *Exact agreement between examiners for medical indication for treatment*

Observers	Agreement (%)	95% CI
All	*60.4*	50.4-69.8
1 vs 2	*78.3*	69.2-85.7
1 vs 3	*66.0*	56.2-75.0
2 vs 3	*76.4*	67.2-84.1

CI = confidence interval.

STUDY II

- Midterm follow-up after endovenous laser ablation of superficial venous incompetence in patients with healed or active venous ulcers achieves good healing

The mean follow up time was 41 months (range 14-89). The mean age at the time of operation was 62.4 ± 11.2 years (mean ± standard deviation) in the C5 group and 64.1 ± 10.5 years in C6 (p = 0.265). The male/female ratio was 42/52 in C5 and 30/46 in C6 group (p = 0.761). Demographics and characteristics of patients/limbs are shown in Table 7.

Table 7. *Demographics and characteristics, comparing subjects with healed and open ulcer at the time of EVLA treatment*

	Total	C5 group*	C6 group**	*P* value
Patients	170	94	76	
DM	14 (8%)	7 (7%)	7 (9%)	0.797
CVD	35 (21%)	17 (18%)	18 (24%)	0.414
Smoker	13 (8%)	5 (5%)	8 (11%)	0.307
No. of Limbs	195	109	86	
EVLA of GSV	158 (81%)	91 (83%)	67 (78%)	0.324
EVLA of SSV	50 (26%)	22 (20%)	28 (33%)	0.049
EVLA of AASV	16 (8%)	11 (10%)	5 (6%)	0.280
EVLA of PV	2 (1%)	2 (2%)	0 (0%)	0.504

The patients' characteristics did not differ between the C5 and C6 groups; therefore limbs from both groups were grouped together to compare parameters according to ulcer recurrence.

The only significant differences between the original C5 and C6 limbs were a slightly longer follow-up (18 days) (p = 0.013) in the C6 group and more limbs operated with EVLA of the SSV (p = 0.049). Limbs with recurrent VU had a statistically significant association with male sex (p = 0.026), lower HRQoL (p = 0.037), higher VCSS score (p < 0.001), reduced ankle mobility (p = 0.047) and impaired venous function (p = 0.029) with lower venous refill times, all in the univariate analysis. Further analysis was conducted to assess risk factors for recurrence with logistic regression. Variables included were perforator vein insufficiency (PVI), VV in the ulcer area, deep venous reflux and reduced ankle mobility. The only variable significantly associated with ulcer recurrence was reduced ankle mobility (p = 0.048).

STUDY III

- There are increased plasma levels of cross-linked fibrin degradation products by leukocyte elastase (E-XDP) with increasing disease severity.

The mean age was 63.53 years, (range 23-89), 81 were females and 61 males. Patient demographics and characteristics are shown in Table 8. C-class was used to group the patients on analysis, C 0-1 were controls, C 2-3 was the second group and C 4-6 the third group.

Table 8. *Patient characteristics and demographics*

	C0-1 (n=10)	**C2-3 (n=41)**	**C4-6 (n=91)**
Age (years), mean (SD)	58.2 (13.9)	61.0 (13.7)	64.6 (13.3)
Gender, n (%)			
Female	9 (90)	27 (65.9)	45 (49.5)
Male	1 (10)	14 (34.1)	46 (50.5)
History of DVT (%)	0	1 (2.4)	9 (9.9)
Use of Statin (%)	0	0	15 (16.5)
Use of anticoagulant (%)	0	6 (14.6)	25 (27.5)

The following clinical features were analysed using univariate analysis before subjection to multiple linear regression: age ($p < 0.001$), gender ($p = 0.708$), use of statins ($p < 0.001$), anticoagulants ($p < 0.001$) and history of DVT ($p = 0.015$). Multiple linear regression confirmed that E-XDP was independently associated with CVD ($p < 0.05$) after adjustment for age and gender (Figure 1). However, after adjustment for differences significant in univariate analysis such as use of statins, anticoagulants and history of DVT, the previous association was not seen between higher concentrations of E-XDP and CVD.

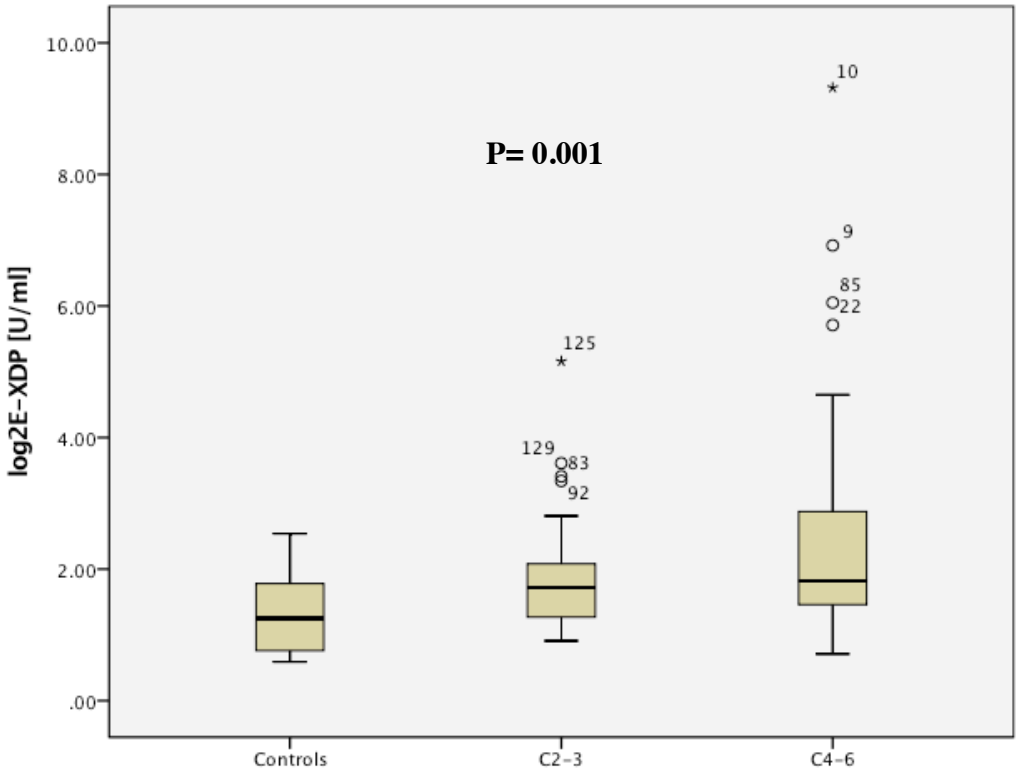

Figure 1. *Concentration of E-XDP between groups of chronic venous disease*

STUDY IV

- The Swedish VEINES-QOL/Sym showed excellent internal consistency for both VEINES-QOL and VEINES-Sym.

The included patients were classified according to the C-class of CEAP and 6% were classified as C1, 46% as C2, 7% as C3, 25% as C4, 9% as C5 and 6% as C6. Missing values for both questionnaires were less than 10%, indicating good feasibility. There were no apparent floor or ceiling effects for the VEINES-QOL/Sym questionnaire.

The mean age was 54.5 ± 15.2 years (range 19 – 83) and patient characteristics are shown in Table 9.

Table 9. *Patient characteristics and clinical data*

Characteristics	**N (112)**	**%**
Gender		
Male	28	25
Female	84	75
Age (years)		
Mean ± SD (range) 54.5 ± 15.2 (19 - 83)		
Previous VV surgery		
Yes	45	40
No	67	60
History of DVT		
Yes	10	8.9
No	102	91.1

The Cronbach's alpha coefficient showed an excellent internal consistency for both VEINES-Sym and VEINES-QOL, measures were $\alpha = 0.89$ and $\alpha = 0.93$ respectively.

The factor analyses showed that the VEINES-Sym items fit the structure well (Table 10). All but item 1h (´during the past 4 weeks, have you experienced itching in the affected leg(s), loading of 0.334´) of the 25 items of the VEINES-QOL fit the structure well. Our results were compared with the Dutch results and the patterns of factor loadings were similar between the Swedish and the Dutch version(98).

The three researchers tested content and face validity and agreed that the VEINES-QOL/Sym covers central aspects of CVD.

Comparing the VEINES-QOL/Sym scores with the RAND-36 subscale scores tested construct validity. The strongest correlation when it came to VEINES-Sym was noted with the RAND-36 subscale, bodily pain (BP) ($r = 0.76$; $p < 0.01$). When it came to the VEINES-QOL, the strongest correlations were noted with the RAND-36 subscales BP ($r = 0.82$; $p < 0.01$) and physical function (PF) ($r = 0.66$; $p < 0.01$).

Table 10. *Factor loadings of an exploratory ordinal factor analysis (SWE)**

Items		VEINES-Sym		VEINES-Sym/QOL	
		SWE	**NL**	**SWE**	**NL**
q1a	**Heavy legs**	.764	0.61	.730	0.54
q1b	**Aching legs**	.882	0.73	.762	0.59
q1c	**Swelling**	.585	0.46	.440	0.38
q1d	**Night cramps**	.570	0.49	.530	0.43
q1e	**Heat or burning sensation**	.677	0.64	.502	0.52
q1f	**Restless legs**	.731	0.65	.652	0.50
q1g	**Throbbing**	.740	0.63	.553	0.58
q1h	**Itching**	.410	0.46	.334	0.40
q1i	**Tingling sensation**	.771	0.68	.601	0.55
q7	**Intensity of leg pain**	.736	0.71	.714	0.62
q3	**Compared to one year ago …rate your leg problem**			.451	0.31
q4a	**Daily activities at work**			.724	0.56
q4b	**Daily activities at home**			.722	0.79
q4c	**Standing for long periods**			.749	0.68
q4d	**Sitting for long periods**			.673	0.66
q5a	**Cut down time spent on work**			.524	0.75
q5b	**Accomplished less**			.678	0.76
q5c	**Limited in kind of work**			.626	0.56
q5d	**Difficulty performing work**			.692	0.67
q6	**Interference with normal social activities**			.775	0.77
q8a	**Concerned about appearance of leg(s)**			.357	0.29
q8b	**Felt irritable**			.660	0.66
q8c	**Felt a burden**			.703	0.55
q8d	**Worried about bumping into things**			.532	0.41
q8e	**Appearance of leg(s) influenced choice of clothing**			.362	0.35

NL; See S K van der Velden et Al: Translation and validation of the Dutch VEINES-QOL/Sym in varicose vein patients. Phlebology 2014; 29: 227–235, Table 2.

To examine known-group differences we hypothesised that disease severity would correlate with poor HRQOL but this was not confirmed. There were no statistically significant differences found between the VEINES-Sym ($p = 0.57$) and VEINES-QOL ($p= 0.22$) mean scores and the severity of CVD even if there was a trend for lower VEINES-QOL scores with increasing severity of CVD classified as worst C-class of CEAP.

GENERAL DISCUSSION

This thesis aimed to highlight some of the challenges on the topic of CVD. Diagnosis and prioritization that are discussed in study I, III and IV are of great importance not only for the health care system/budget but also for the suffering patients. Evaluation of a relatively new treatment method for VU patients and introduction of a disease-specific patient reported outcome measure (PROM) in Swedish as discussed in studies II and IV further highlight crucial needs in the field of CVD.

The basic CEAP classification is the recommended gold standard for documentation of CVD as it facilitates diagnosis and prioritization of the disease(53, 57). In study I where we investigated its use in clinical practice we saw that assessments differed considerably for both clinical class of CEAP and assessment for the medical indication for treatment between the investigators. Similar results were found in a recent study by Lattimer et al, where clinicians graded photographs of CVI patients according to the clinical class of CEAP(99).

When analyzing the C classes separately, some difficulties stood out. Least agreement was seen for C3, although the number of patients was small. However when discussing the study results, it was obvious that we all found defining edema difficult. The definition of edema in the revised version of CEAP is "*perceptible increase in volume of fluid in skin and subcutaneous tissue, characteristically indented with pressure. Venous edema usually occurs in ankle region, but may extend to leg and foot.*"(76) Some patients in the study had minor edema in the ankle region indented by pressure from a sock, but this could also be considered physiological from prolonged standing; thus, the leg would be classified as C2 instead of C3, edema. Another surprising difficulty, even if described previously, was in distinguishing widespread telangiectasies from hyperpigmentation i.e. C2 from C4(100).

In order to make the grading of venous symptoms more reliable, methods such as quality of life questionnaires and photoplethysmography have been used, but it has not been proven in larger studies that these methods really make the evaluation more consistent(68, 101).

The discrepancy in classifying according to CEAP brings to light two separate problems: scientific studies using the CEAP may not be reliable if the participating doctors are classifying differently, and in clinical daily life, patients may not be evaluated in an equitable way when it comes to reimbursement.

In spite of the results in study I, the CEAP classification is the gold standard and necessary for prioritizing of patients.

Endovenous ablation techniques have largely replaced open surgery of VV and in study II we try to evaluate mid-term outcomes on one of these techniques, namely EVLA. The National Institute for Health and Care Excellence has formed guidelines recommending minimally invasive techniques including EVLA, RFA and UGFS before surgery for treatment of VV and these guidelines have been challenged(102, 103).

In study II where the patient cohort includes patients with healed or active VU treated with EVLA for SVI, our results show that 84% of the treated legs still had a healed ulcer without recurrence after 3.5 years in a group of 170 patients where 16% were lost to follow-up. Complications were mostly minor and 15% had undergone repeat treatment of varicose veins. The study thus confirmed the findings of previous smaller studies with shorter follow-up(104). Many of the patients included were elderly with concomitant diseases, and we hope that the results of this and similar studies can encourage treating this particular group of patients more actively.

Deep venous reflux, presence of PVI and/or varicose veins in the ulcer area and reduced ankle mobility are considered risk factors for ulcer recurrence(105, 106) and in this study this was confirmed in the comparison of groups but not in the logistic regression except for reduced ankle mobility.

The role of PVI remains uncertain, there are no randomized trials with long-term follow-up showing that treating PVI reduces VU recurrence, even though several other studies have shown low recurrence rates(100, 107, 108). Presumably, patients with active or previous VU are more vulnerable even for modest remaining venous reflux in the area, PVI or other, and thus could benefit more from radical treatment than VV patients without skin changes.

A remarkable finding in study II was that all C6 legs had healed their original VU at some time before the study examination, after which it recurred in 14 patients. In most reports a certain percentage will not heal unless complete eradication of venous reflux and on-going ambulatory compression therapy. This result may be due to patient selection, presumably patients with giant ulcers of long-standing duration were not referred to the clinic and few patients with a history of DVT were included.

The strength of this study is that EVLA was performed in clinical practice with no special care or follow-up for VU patients and even so the recurrence rate was low.

A limitation with the study was that we did not know how much the added treatments of local varicosities and PVI improved the long term results in terms of ulcer healing and recurrence, and in the medical records it was not specified exactly how many perforators and VV in the ulcer area were treated.

CVD being an inflammatory disease, markers such as albumin, fibrinogen, d-dimer and leucocyte counts have been studied to show if they can be used as predictors of progression of CVI(109). In study III, a cross-sectional study, we looked at E-XDP levels in patients with CVD. We found that these patients have elevated levels of circulating E-XDP compared to controls. This association between CVD and E-XDP was confirmed after adjustment for age and gender but we have no evidence to conclude that such an association exists after adjusting for use of statins, anticoagulants and history of DVT.

On comparison between groups with increasing severity of CVD, there was a significant difference between groups but the reason for the elevated E-XDP is unknown. It may be that the patients have thrombi in other parts of the body or micro thrombi in the vein wall that are also degraded by leucocyte elastase but other unknown sources of thrombi can not be neglected(110, 111). On multivariate analysis and after adjusting for use of statins, anticoagulants and history of DVT, there was no statistically significant association between E-XDP and CVI. Possible explanations to this is small sample size, too few controls or that there is no actual association between CVI and elevated levels of E-XDP.

The main limitation with the study was that none of the controls were on statins or anticoagulants. Ideally we should have matched for age and for medications.

Another limitation was that we do not know if E-XDP levels increase over time with increasing disease severity. Therefore we need to follow these patients over time with further analysis. The most interesting patient group to follow would be C2 patients with elevated E-XDP levels to see if they develop skin changes over time.

In the fourth study our results demonstrate that the Swedish version of VEINES-QOL/Sym, a disease specific HRQOL questionnaire for venous disease, is a valid and reliable scoring instrument for patients with CVD. The reliability was well supported by levels of Cronbach's alpha above 0.8 for VEINES-QOL/Sym and there were significant correlations between all the RAND-36 subscales and the VEINES-QOL/Sym scores. These results are similar to findings in the original study and others(112-114).

The C-class of the CEAP-classification had no significant correlation with the VEINES-QOL or VEINES-Sym scores but there was a trend for poorer outcome for VEINES-QOL with increasing disease severity. The reason for this finding raises the question whether it is the CEAP classification with inherent difficulties as discussed in study I or problems with the VEINES-QOL/Sym. Despite this result we still believe that PROMs are necessary as a complement to clinical severity scores such as VCSS.

In our study, we used the RAND-36 questionnaire, which is similar to the SF-36 except for differences in the scoring of general health and bodily pain, and in Swedish RAND-36 has a slightly modernized wording(115, 116). Our results concur with the original study with regard to construct validity(112). Generic instruments such as the RAND-36 are regularly used in venous disease studies and in clinical practice but disease specific instruments have the superiority of differentiating between diseases(55).

The exploratory factor analysis was consistent with the findings in the Dutch study with similar factor loading patterns(98). Only one item loaded below the cut-off of 0.35 but since the reliability analysis didn't show a low alpha value, we chose to keep the item.

A limitation of our study was that we weren't able to assess test–retest reliability of the VEINES-QOL/Sym, which is an indicator of the stability of a measuring instrument.

METHODOLOGICAL CONSIDERATIONS

All the studies included in this thesis are observational studies as opposed to experimental studies.

Observational studies (OS) are the kind where the researcher systematically collects information but does not alter what occurs(117). Types of OS include cohort, cross-sectional and case-control studies.

Cohort studies allow you to study changes and to establish the time-sequence in which things occur. They are like cross-sectional studies but extend over time. This permits study of cause. Disadvantages of this type of study are the need for a large sample size and very long follow-up times that are not only time consuming but also increase the risk of losing subjects to follow-up.

In **cross-sectional studies** (Studies I, III and IV) you draw a random sample of people and record information about their health in a systematic manner. This type of study allows for comparison between study subjects with and without the disease. A disadvantage of this study type is causation; you do not know what came first.

Case-control studies are more practical studies but are retrospective which means many disadvantages. The obvious advantage is that this type of study can be done faster and more cheaply than a cohort study. However, it may be difficult to collect the information you require on past exposures, and there may be other ways in which the cases and controls differ. Ways of avoiding this is by ensuring greater comparability between the two groups, and thereby avoid confounding bias; the controls could be matched for sex and age to the cases.

Types of bias that can occur in OS are confounding and selection bias. **Confounding** occurs when there is a failure to adjust for common causes of both the exposure and the outcome(118). In both studies II and III this was an issue that we tried to adjust for by using multivariate analysis. During the selection of predictors to include in the multivariate regression models, we included well established or probable causal factors and factors considered as clinically relevant. This was done without regard to statistical significance or strength of their association with the predictor of primary interest and outcome.

In study II and III there was a difference between the univariate and multivariate analysis. The explanation was probably multi-factorial, collinearity between predictors in the multivariable regression and lack of power to detect a statistically significant association. In study III more confounders could have been included like other comorbidities including diabetes, heart disease or arterial disease, as these could be residual confounders that can never completely be ruled out.

Selection bias is the systematic error that results from the way subjects are selected into the study or because there are selective losses of subjects before data analysis(119). This kind of bias together with confounding is the main obstacle for high validity in a study. In cohort studies, the primary sources of selection bias are loss to follow-up, withdrawal from the study, or nonresponse. Studies II and III were both vulnerable for this bias.

In study II, patients were identified from the clinics quality registry, procedure codes for EVLA and diagnosis codes for venous ulceration in the medical records. Between the time of surgery and time of study the clinic changed it's journal system, to minimize the risk of losing patients between system changes, we searched according the criteria named above in both old and new journal systems but this wouldn't guarantee losing patients to misclassification. Misclassification in this case would be patients with VU but treated and given a surgical code other than EVLA or patients wrongly diagnosed. Other reasons for bias could be either that the patients suffered complications and sought care else where, or recurred in ulcer disease but chose not to come back to the clinic or moved away and for that reason could not be invited for follow-up.

In study III most patients were elderly and we had a small control group so these facts could affect our findings. In cross-sectional studies, the primary source of selection bias is "selective survival." Study III could theoretically suffer from this bias but since near to none patients die from CVD, this is a bias we didn't have to take into consideration in our study.

CONCLUSIONS

The present thesis has demonstrated that:

- The reproducibility of the clinical class of CEAP when deciding medical indication for treatment is generally moderate. This may be due to inherent difficulties in the CEAP, lack of specific training, or the simultaneous assessment of reimbursement that may influence the clinical classification.
- Endovenous laser ablation of superficial venous insufficiency in patients with healed or active venous ulcers confers both ulcer healing and low recurrence rates in a majority of patients with a low rate of complications and a modest rate of re-interventions.
- There is an association between elevated levels of E-XDP and increasing disease severity in CVD.
- The VEINES-QOL/Sym-Swe is a valid HRQOL instrument for CVD and confirms previous studies validating the original English version.

FUTURE PERSPECTIVES

CVD and its most severe form VU probably consume more resources from society than funds allocated to research within the field. The "Cinderella" of surgery has despite that managed to intrigue my curiosity and further broaden my knowledge on the subject. The prevalence of CVD doesn't seem to be decreasing and globally we are becoming more aware of its scope and need for further epidemiological studies. With this background, it is obvious that future studies are a necessity.

Our findings in study I invite for a study where we train the investigators in the C-class of CEAP prior to a similar study as study I. Another kind of study would be one where the investigators weren't required to make a simultaneous decision on medical indication for reimbursement while grading with CEAP.

Treatment with EVLA showed good mid-term ulcer recurrence results but a randomized controlled study comparing open surgery vs EVLA and compression vs EVLA may better answer the question about the efficacy of endovenous methods in treating patients with healed or active venous ulcers.

Our study on a potential biomarker as a predictor of presence or disease severity causes speculation that the increased levels of E-XDP increase over time with increasing disease severity. A longitudinal follow-up study where E-XDP and other biomarkers are taken in C2 patients with elevated E-XDP and following them to see if they develop VU could possibly answer this question.

Further, with emerging new treatments and evaluation of their effects, quality of life instruments are an appropriate way of doing so. In Sweden there is currently no disease-specific HRQoL instrument and after translation of the VEINES-QOL/Sym into a Swedish version, we see the need to modify it into a short version that is more convenient in clinical practice. This will also allow for more studies on CVD where a disease-specific instrument is available. A shortcoming with our study was that we didn't assess test–retest reliability of the VEINES-QOL/Sym. We therefore plan a later study to answer the question of reproducibility. In addition, we found poor correlation between the VEINES-QOL/Sym and CEAP, which has its shortcomings as described above, and this brings reason to study the correlation between VEINES-QOL/Sym and duplex or PPG findings.

SAMMANFATTNING PÅ SVENSKA

Kronisk venös sjukdom (CVD) är så vanligt förekommande att gränsen mellan normalvariant och sjukdomstillstånd är flytande. Enligt moderna studier har c:a en tredjedel av en västerländsk befolkning varicer. Benämningen kronisk venös insufficiens (CVI) hänvisas till den mer avancerade formen av CVD och yttrar sig dels som venösa hudförändringar såsom eksem, missfärgning och inflammationer vilket förekommer i 7 % och dels som bensår och förekommer i 1 % av alla med CVD. Omkostnaderna för bensår (varav venösa bensår är i majoritet) beräknas till 1-2 % av EUs totala hälso- och sjukvårdsbudget. Venösa bensår är ofta smärtsamma och långvariga, och medför höga personalkostnader pga. omläggningar och kompressionslindning.

Den klassifikation som används internationellt för att klassificera venös sjukdom, den kliniska C-delen av CEAP (Clinical Etiology Anatomy Pathophysiology), ligger till grund för landstingets rekommendation av vilka varicer som ska opereras på medicinsk indikation men är dåligt utvärderad trots att den används i nästan alla vetenskapliga studier om venös insufficiens.

Det senaste decenniet har det blivit allt vanligare att traditionell åderbråckskirurgi ersätts med lasermetoder där åderbråcken bränns inifrån istället för att tas bort, s.k. endovenös laser. Metoden har vissa fördelar då den kan utföras mer skonsamt och därmed kan även äldre och sköra bensårspatienter behandlas. Enligt nyliga studier är metoden troligen likvärdig på sikt med vanlig kirurgi, men konvalescensen är kortare. Det är dock inte undersökt om själva läkningsprocessen för venösa bensår påverkas.

I denna avhandling var övergripande målet att undersöka möjligheter att förbättra prioritering samt utredning och kirurgisk behandling av patienter med åderbråck som har eller löper risk att utveckla venösa sår. Mer specifikt undersöktes (I) om CEAP-klassifikationen kan användas med god överensstämmelse mellan olika bedömare, (II) medför variceroperation med endovenös laser varaktig sårläkning på patienter med venösa sår, (III) om markören XDP i plasma skiljer sig mellan patienter utan venös sjukdom och med olika grad av hudförändringar vid kronisk venös insufficiens och (IV) om kan man översätta och validera ett sjukdomsspecifikt

livskvalitetsformulär från engelska till svenska, nämligen VEINES-QOL/Sym. Resultat från studierna som ingick sammanfattas nedan.

Studie I visade att när tre av varandra oberoende kirurger klassificerade 106 ben på 78 patienter avseende klinisk svårighetsgrad enligt CEAP klassifikationen och avseende medicinsk indikation för ingreppet förelåg endast en måttlig överensstämmelse.

Studie II visade att 84 % av 170 patienter (195 ben) har läkta bensår utan recidiv 3,5 år efter operation och med få komplikationer. Vår studie speglar den kliniska verkligheten med positiva resultat. Nedsatt rörelseförmåga i vristen var en signifikant riskfaktor för recidiv av bensår i vår studie som tidigare visat.

Studie III visade att det finns en koppling mellan förhöjda plasma nivåer av E-XDP och CVD. För att kunna använda E-XDP som biomarkör i diagnostiskt syfte av CVD patienter kräver dock ytterligare studier.

I studie IV visade resultaten att det finns en utmärkt intern tillförlitlighet för både VEINES-QOL och VEINES-Sym. Båda VEINES-QOL och VEINES-Sym korrelerade väl till alla domän i RAND-36 (generisk livskvalitetsformulär), demonstrerande god konstruktions validitet.

Sammanfattningsvis påvisar resultaten i denna avhandling att C-delen av CEAP är fortsatt gold standard vid diagnostisering av CVD trots godtycklig reproducerbarhet och bensårs patienter som behandlas med EVLA får en varaktig sårläkning med få komplikationer. Vidare verkar E-XDP ha en positiv korrelation med ökande grad av CVD. Sist men inte minst, Svenska versionen av VEINES-QOL/Sym är en validerad livskvalitets instrument för CVD och kan användas kliniskt och för forskning.

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